Abstract

Obesity and diabetes are metabolic complications associated with higher accumulation of fat in periphery and bones leading to higher fracture risk. Several approaches have been introduced in the treatment of obesity and diabetes - non-pharmacological (e.g. dietary intervention or physical activity) and pharmacological (e.g. metformin, thiazolidinediones (TZDs)) to improve metabolic complications.

Dietary intervention including omega-3 polyunsaturated fatty acid (PUFA) supplementation showed multiple beneficial effects on bone parameters and bone marrow skeletal stem cells (BMSC) properties in animal and clinical studies. However, this was not well studied in obesogenic conditions. Further, TZDs (e.g. pioglitazone, rosiglitazone) are very effective insulin sensitizers but with several side effects (increased bone marrow adiposity (BMA) with higher fracture risk) due to their mechanism of action. MSDC-0602K, a novel TZD analog, has been developed to minimize side effects on fat metabolism. However, its impact on bone phenotype and bone marrow skeletal stem cells BMSCs in relation to obesity has not been intensively studied.

Thus, in my PhD thesis I investigated both approaches using high-fat diet (HFD)-induced obesity mouse model. 8 weeks old male mice were fed with HFD or HFD supplemented with omega-3 PUFA, MSDC-0602K or pioglitazone for 2 months to investigate their effects on metabolic, bone parameters and stem cell properties using several methods including µCT, histology, analysis of osteoclast differentiation from bone marrow (BM) cells isolated from treated mice (in study using omega-3 PUFA treatment), cultivation of primary BMSCs and their cellular and metabolic characterization.

In dietary interventional study, omega-3 PUFA treatment showed robust changes in bone microstructure and mechanical properties characterized by low cortical porosity and BMA in tibia, and increased bone strength of femur compared to HFD. These changes were also manifested on cellular level, by decreased osteoclastogenesis of BM cells, increased osteoblast differentiation of BMSCs coupled with decreased cellular respiration and lower senescent phenotype with omega-3 PUFAs. Further, pharmacological approach comparing classical TZD pioglitazone with MSDC-0602K showed less detrimental effect of new analog on bone microstructure compared to pioglitazone group, which was accompanied with higher distribution of smaller BM adipocytes. Cellular analysis revealed increased osteoblast differentiation of primary BMSCs in MSDC-0602K treated mice. However, cellular respiration

as well as glycolysis were increased in MSDC-0602K BMSCs compared to pioglitazone group suggesting different utilization of nutrients by these drugs. These unexpected results led us to compare effect of these drugs on bone and peripheral AT metabolism using BMSC cell line and adipose derived stem cell line (AT-MSCs) 3T3-L1, where we observed decreased respiration with MSDC-0602K compared to pioglitazone, suggesting also different mechanism of action in bone and in periphery. To further understand the TZD actions on cellular metabolism in periphery and bones, we measured nutrient utilization in BMSC cell line and AT-MSCs. We observed increased utilization of glutamine by MSDC-0602K in BMSCs compared to pioglitazone, while in AT-MSCs MSDC-0602K preferred glucose over glutamine. Thus we performed fluxomic analysis of ^{13}C labelled glucose and glutamine in BMSC treated with MSDC-0602K and pioglitazone and we observed increased glutamine and decreased glucose utilisation with MSDC-0602K compared to pioglitazone. These results are supporting our hypothesis that increased glutamine metabolism in BMSCs by MSDC-0602K treatment can contribute to lower senescence, increased osteoblastogenesis of BMSCs and improved mechanical properties of bones.

Taken together, our findings using non-pharmacological treatment in obesity showed pronounced beneficial effects of omega-3 PUFA supplementation of HFD-treated mice on bone microstructure, bone mechanical properties and cellular homeostasis. Further, pharmacological approach showed decreased side effect of treatment on bone and BMSC properties with MSDC-0602K compared to pioglitazone by activation of glutamine metabolism. Thus, this thesis brings two potential strategies of preventing negative effect of metabolic diseases but also opens a new perspective for further research of synergistic effect of these molecules in clinical practice.

Keywords: Obesity-induced bone fragility; Omega-3 polyunsaturated fatty acids, Thiazolidinedione analog MSDC-0602K; Bone marrow stromal cells; Cellular metabolism; Bone marrow adipose tissue; Bone microstructure